

## XL. THE NATURE OF YEAST FAT.

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COMPARATIVELY little is known of the nature of the fat present in simple cellular organisms and of the processes by which it is formed. It is possible that a study of such organisms, where the conditions of growth are easier to control, may throw more light on the method by which fat is formed than investigations of the more complex plants and animals. Since yeast is obtainable in quantity, it was chosen as the most suitable material for such an investigation; as a preliminary it was necessary to identify with certainty the constituents of yeast fat.

### CHARACTERISTICS OF YEAST FAT.

The fat examined was obtained from the following sources:

- (1) Baker's yeast supplied from the manufacturers.
- (2) A pure culture of a brewery yeast grown for us under definite conditions by Dr Thaysen at the bacteriological laboratory of the Royal Naval Cordite Factory.
- (3) Specimens of brewery yeast.

### *Method of Extraction of the Fat.*

The pressed yeast was spread in a thin layer and dried in the hot room at 37° for 48 hours<sup>1</sup>. It was then soaked over night in alcohol and shaken for eight hours at ordinary temperature. The second and third extractions were similarly carried out, a mixture of alcohol and light petroleum (B.P. 60–80°) being used as the solvent. After the third extraction, 25 g. of the dry residue were extracted for eight hours in a Soxhlet apparatus with ether. The amount of fat obtained in the final extraction was always very small and was used only for calculating the total amount of fat. The alcohol and petroleum were evaporated on a water bath under diminished pressure and the residue dissolved in light petroleum and filtered. After evaporation of the solvent, the residue was dissolved in alcohol and excess of acetone added to precipitate the lipins. The solvent was distilled from the filtered solution; the iodine and saponification values were determined and the fat converted to the methyl esters, or saponified for the preparation of the sterols.

<sup>1</sup> Recently, the preliminary drying at 37° has been discarded since a considerable proportion of the fat seems to disappear during the process.

The crude yeast fat prepared by extraction of the alcohol-soluble residue with ether or with light petroleum consists of a brown liquid from which crystals of a sterol separate on standing. It has a high iodine value and a low saponification value attributable to the large proportion of unsaponifiable matter present which may be from 25 to 45 %.

#### *Iodine Value.*

The iodine value was determined by Wijs' method after precipitating the lipins by acetone. Twelve specimens grown from a pure culture of brewery yeast by Dr Thaysen gave values lying between 147 and 175.5, the average being 161.2. Four specimens of brewery yeast obtained locally gave an average value of 164.4. Six specimens of baker's yeast gave values from 121.2 to 150.8, the average being 135.6.

The iodine value therefore varies within wide limits. We found that this was dependent chiefly on the amount of sterol present in the crude fat. The yeast sterol has a very high iodine value and gives quite erratic results when the latter is determined by Wijs' method (see p. 490).

#### *Saponification Value.*

This is always low and shows wide variation, depending on the proportion of sterol present. Nine specimens of brewery yeast obtained from Dr Thaysen gave an average value of 162.3, the numbers lying between 138 and 184; four specimens of brewery yeast obtained from a brewery gave an average value of 164. The values from baker's yeast were higher, three specimens from one firm giving values from 196 to 199, while other specimens gave values of 187 and 151.

We endeavoured to determine the proportion of sterol in a specimen of crude fat obtained from baker's yeast, and in the unsaponifiable matter derived from it, by precipitation with digitonin solution [Windaus, 1909]. The result shows that the greater proportion of the sterol seems to be present not in the free state but as fatty acid ester.

In one experiment, the proportion of unsaponifiable matter was determined by extracting the solution with ether after it had been saponified and drying the residue in vacuo at 100°.

0.5456 g. fat gave 0.2175 g. unsaponifiable matter.

The unsaponifiable residue from a similar quantity of fat was dissolved in 50 cc. 95 % alcohol and a solution of 0.5 g. digitonin in 50 cc. of 90 % alcohol added. The resulting precipitate was filtered through a Gooch crucible, washed with 95 % alcohol and dried at 110°.

Weight of digitonin cholesteride 0.3763 g.

Solubility allowance 0.014

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0.39 g.

% sterol = 17.23 %.

0.266 g. fat dissolved in 90 % alcohol and precipitated with 25 cc. of a 1 % solution of digitonin in 95 % alcohol gave 0.051 g. or allowing for solubility = 0.058 g.

% unsaponifiable matter	= 39.8 %	} 17.2 %.
% combined sterol	= 12.3 %	
% free sterol	= 4.9 %	

Yeast sterol very rapidly undergoes alteration; some decomposition appears to take place during the saponification process and it seems probable that the figures obtained for the sterol are too low. It is also probable that the solubilities of the yeast sterol and the cholesterol compounds with digitonin are not identical; the figures given can therefore only be regarded as approximate, and as representing the minimum amount of sterol in the sample.

#### CONSTITUENTS OF YEAST FAT.

##### *The Fatty Acids.*

Nägeli and Loew [1878] first investigated yeast fat and described its fatty acid as consisting mainly of oleic acid. Gérard and Darexy [1897] found in it butyric, palmitic and stearic acids. Hinsberg and Roos [1903] isolated from it three fatty acids, (1) an acid identical or isomeric with oleic, (2) an unsaturated acid having the formula  $C_{12}H_{22}O_2$ , and (3) a saturated pentadecic acid to which they ascribed the formula  $C_{15}H_{30}O_2$ . These observers noted the ready oxidisability of the acids, in one instance the bulk of the acid having been converted to a neutral substance after standing for three weeks. The following year [1904] they succeeded in isolating and identifying a specimen of palmitic acid from the alleged pentadecic acid.

Neville [1913], apparently in ignorance of the later work of Hinsberg and Roos, confirmed the presence of the acid  $C_{15}H_{30}O_2$  which he was unable to separate into palmitic and a lower acid. He also isolated a substance melting at  $77^\circ$  which he regarded as arachidic acid; from a study of the oxidation products of the unsaturated acids he deduced the presence of an acid isomeric with linolic acid and of the  $C_{16}$  and  $C_{18}$  members of the oleic series.

We used as material for this part of the investigation, baker's yeast containing 2 % of fat calculated on the dry yeast. This had an iodine value of 121.2 and a saponification value of 187.5.

##### *Preparation of the Methyl Esters.*

Haller's method, *i.e.* heating with a 2 % solution of HCl in methyl alcohol, was first tried but is unsuitable when dealing with a very unsaturated fat such as that of yeast. The fat is little soluble in methyl alcohol: prolonged heating is necessary to bring about alcoholysis and during this time a heavy brown insoluble layer forms. These results resemble those obtained by Haller [1905] when working with linseed oil. Bull's method [1906] was therefore adopted.

100 g. of fat were dissolved in light petroleum and shaken with 26.18 cc. of a 4.25*N* solution of sodium methoxide in methyl alcohol. After standing two hours, water and light petroleum were added and the upper layer separated, washed with sodium carbonate solution and dried with  $\text{CaCl}_2$ . After distilling off the solvent, the methyl esters were twice distilled under 20 mm. pressure with a six-pear distilling column. The following fractions were obtained:

B.P.			
145–155°	...	...	1.1 g.
155–180°	...	...	0.2 g.
180–195°	...	...	2.3 g.
195–200°	...	...	9.0 g.
200–205°	...	...	0.5 g.
205–210°	}	...	21.0 g.
210–215°			
215–220°	...	...	6.0 g.

There remained a considerable brown residue in the flask after the first distillation which decomposed on further heating.

#### *Identification of Saturated Acids.*

The fraction 195–200° partly solidified on standing; the solid ester was separated, pressed out, hydrolysed and the acid recrystallised from alcohol. After nine recrystallisations an acid melting at 61–62° was obtained, the melting point not being raised by further recrystallisation.

Analysis showed this to contain 75.00 % C and 12.28 % H. Calculated for palmitic acid 75.00 % C and 12.5 % H. The fraction boiling from 145 to 155° had an iodine value of 61; it was hydrolysed and the acid recrystallised, a solid acid separated which after recrystallisation melted unsharply at 38°. Methyl laurate boils at 148 under 18 mm. pressure; lauric acid melts at 43.6°. It seems probable therefore that this acid is lauric acid, but there was not sufficient material to confirm this further. Where only a small proportion of the  $\text{C}_{12}$  acid is present, it is difficult to remove the linoleic and oleic acids completely by distillation, unless large quantities of the acids are available. It may perhaps be regarded as confirmatory evidence that the melting point of the pentadecic acid given by Hinsberg and Roos [1903] as 56° agrees well with that for a mixture of 20 % lauric and 80 % palmitic acid, 57.4° [Heintz, 1854]; the analytical figures they give also agree with those required by such a mixture (cp. Table I). A comparison of the results obtained by the various observers is shown in Table I.

Table I. Saturated fatty acid isolated from yeast fat.

Observer's name	M.P.	% C.	% H.	M.P. Methyl Ester
Hinsberg and Roos (1903)	56°	74.5	12.3	
" " (1904)	62°	74.85	12.67	
Neville (1913)	59°	74.59	12.38	26
Gérard and Darexy (1897)	60–61°			
MacLean and Thomas	61–62°	75.00	12.28	
Palmitic acid	62°	75.00	12.5	28
Pentadecic acid	53°	74.38	12.39	
Mixture 20 % lauric and 80 % palmitic acids	57.4°	74.4	12.4	

The presence of palmitic acid is therefore definitely established and Neville's pentadecic acid is probably a mixture of palmitic with a small amount of lauric acid.

In agreement with Neville's work, from the highest boiling fraction of methyl esters we isolated after hydrolysis a small amount of an acid melting at 77°: this was regarded by Neville as arachidic acid.

#### *Identification of Unsaturated Acids.*

	B.P.	Iodine value
Fraction of yeast fat methyl esters	200–205° (20 mm.)	96
" " "	205–215° "	130
" " "	215–220° "	123
Methyl oleate	212–213° (15 mm.)	85·8
Methyl linolate	207–208° "	172·7

It follows from the boiling points and iodine values of the distilled methyl esters that the bulk of the yeast fatty acids consists of a mixture of linoleic and oleic acids. Bromination of the free acids gave a mixture of bromides totally soluble in ether. From this, a solid bromide was obtained, insoluble in light petroleum, which after being twice recrystallised from alcohol melted at 113–114°. It was therefore identified as tetrabromo-linoleic acid. The solution in light petroleum contained liquid dibromo-oleic acid.

Hinsberg and Roos [1903] isolated a small amount of an acid which from its analytical numbers they regarded as an unsaturated dodecenic acid. It was separated from the ether-soluble lead salts, which, as has been pointed out by Lewkowitsch [1913], contain also small amounts of the  $C_{12}$  and lower saturated fatty acids if these are also present in the mixture. The iodine value of the acid was not determined and the evidence for the existence of a naturally occurring dodecenic acid cannot yet be regarded as convincing. One of us [Smedley, 1912] noted on distilling the esters of the butter fatty acids a rise in the iodine value of the fraction corresponding to the  $C_{12}$  acids and suggested as a possible explanation the presence of a dodecenic acid in butter fat. Crowther and Hynd [1917] distilled a mixture of oleic acid with a mixture of saturated fatty acids and found under these circumstances a curve similar to that obtained with the butter fat esters, giving a perceptible increase of iodine value for the  $C_{12}$  fraction. The presence of an unsaturated  $C_{12}$  acid in yeast, while therefore not definitely excluded, must be regarded as doubtful.

#### LECITHIN.

The presence of lecithin in yeast fat was first demonstrated by Sedlmayer [1903] who described it as dipalmitocholine-glycerophosphoric acid. He obtained 4 % crude lecithin calculated on the dried yeast, and estimated the amount of pure lecithin as 2 %. Since the whole of the ether-soluble fraction, consisting mainly of sterol and fat, was regarded as the crude lecithin, these figures are too high. We found that the fraction precipitable from ether or alcohol solution by the addition of acetone gave an average value of 8·5 % of the total fat or about 0·17 of the weight of dried yeast.

## THE STEROL OF YEAST.

Gérard [1895] isolated from yeast a plant cholesterol melting at 135–136° and regarded it as belonging to the same group as the ergosterol obtained by Tanret [1889] from ergot. The latter substance however melted at 154°. Gérard regards ergosterol as typical of a group of sterols characteristic of the cryptogams and differentiated by their reaction with  $\text{CHCl}_3$  and concentrated  $\text{H}_2\text{SO}_4$  from the phytosterols and cholesterol characteristic of the higher plants and animals respectively. The latter substances when concentrated sulphuric acid is added to their chloroform solutions, impart a red colour to the chloroform solution, the acid layer becoming fluorescent. With ergosterol however under similar conditions the chloroform solution remains colourless or shows a slight green fluorescence, the acid taking on a deep red colour.

Hinsberg and Roos [1903] isolated two sterols one melting at 159° and one at 148–149°; the melting point of the latter they were unable to raise by recrystallisation. They state that the sterol of m.p. 159° gives a red colour with chloroform and concentrated acid but do not say which layer contains the red colour. They found that their sterol was not identical with the caulosterol (m.p. 158–159°) isolated by Barbieri and Schulz [1882]. Analysis of the sterol after drying over sulphuric acid gave them results agreeing with the formula  $\text{C}_{26}\text{H}_{44}\text{O}$ . Neville [1913] who next investigated this substance, isolated only a sterol melting at 148–149°.

*Preparation of Yeast Sterol.*

The fat from baker's yeast and from the brewer's yeast supplied by Dr Thaysen, extracted as described above, was saponified with alcoholic potash, the solution neutralised and extracted with ether, the ether-soluble fraction being recrystallised from alcohol and finally from ether; crystals of the sterol also separated on allowing the fat to stand in the cold.

By repeated recrystallisation of the sterol from ether, we obtained a substance melting at 154°, which appears to be identical with the ergosterol isolated by Tanret from ergot [1889, 1908].

Our yeast sterol when first isolated melted at 135–136° and many recrystallisations were necessary to raise the melting point to 154°. The melting point of ergosterol is given by Tanret as 154° “à l'état brut” and 165° on the Maquenne block. Solutions of yeast sterol become yellow and decompose slightly on heating or even on standing at ordinary temperature, a yellow oily substance being formed. It is therefore difficult to obtain in the pure state by crystallisation, and the discrepancies in the melting point between the results of the specimens of the yeast sterol described by different observers are probably due to this cause. On adding concentrated sulphuric acid to a solution of yeast sterol in chloroform, the acid layer is coloured red. The close agreement in melting point and specific

Table II. Properties of sterols isolated from Cryptogams.

Name	Formula ascribed	Source	% C. (anhydrous)	% H (11.06 85.0 · 11.20 84.64 11.25)	Melting-point	Rotation in CHCl <sub>3</sub> in ether [α] <sub>D</sub>	M.P. acetate	Rotation acetate [α] <sub>D</sub>	Observer
Ergosterol	C <sub>28</sub> H <sub>46</sub> O, H <sub>2</sub> O	Ergot			154°	-114°	169-175°	-80°	Tanret [1889]
"	C <sub>27</sub> H <sub>44</sub> O, H <sub>2</sub> O	"			154° à l'état brut 165° sur le Ma- quenne bloc	-126°	180.5°	-91.8°	" [1908]
"	C <sub>24</sub> H <sub>40</sub> O, H <sub>2</sub> O	"			150°	-89.5 at 15°	165		Ottolenghi [1906]
Yeast sterol		Yeast (brewery)			135-136°	-105°			Gérard [1895]
"	C <sub>28</sub> H <sub>44</sub> O	"	83.35	11.9	(a) 159° (b) 148-149°				Hinsberg and Roos [1903]
"		"			156-157°				Meisenheimer [1915]
"		Yeast			145-147°				Neville [1913]
"		Yeast (a) Baker's (b) Brewery			154° 154°	-117° at 18°	170.5	-87.3°	MacLean and Thomas
Mycosterol	C <sub>30</sub> H <sub>48</sub> O <sub>2</sub>	<i>Collybia shiitake</i>	(not dehydrated) 82.00	10.81	159-160°	-129.4°	169°		Ikeguchi [1919]
"	"	<i>Armillaria edodes</i>	81.87	11.35	159-160°	-129.3°			" "
"	"	<i>Hudnum asparatum</i>	82.02	10.95	159-160°	-129.5°			" "
Fungisterol	C <sub>28</sub> H <sub>46</sub> O, H <sub>2</sub> O	Ergot			144° sur le Maquenne bloc	-22.4°	158.5°	-15.9° (ether)	Tanret [1908]

<sup>1</sup> Solvent not stated.

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rotation, of both yeast sterol and its acetate with the corresponding compounds of ergosterol is shown in Table II. Tanret showed that in ergot the ergosterol is accompanied by a sterol of formula  $C_{25}H_{40}O$ ,  $H_2O$ , m.p.  $144^\circ$  (determined on the Maquenne block) to which he gave the name of fungi-sterol. It is possible that this is also present in yeast but we were unable to detect it.

In one specimen of yeast obtained from a local brewery no ergosterol could be detected but from the unsaponifiable matter a sterol melting at  $97-98^\circ$  and giving a specific rotation of  $+10.1^\circ$  in chloroform solution was isolated. The presence of a positive rotation and the low melting point suggest a resemblance to coprosterol (m.p.  $99^\circ$  and specific rotation  $+24^\circ$ ) which is obtained by reduction of cholesterol in the intestines. It is possible that in this particular specimen reduction of the ergosterol had taken place giving a coprosterol-like compound. We hope to be able to obtain further information on this point.

#### *The Iodine Value of the Sterols.*

As stated above in the discussion of the iodine value of yeast fat, very high iodine values were obtained on treating yeast sterol with Wijs' reagent. The fact that the iodine value of cholesterol when determined by Wijs' method varies with the time of the reaction and with other conditions has been noted by Werner [1911] and by Lewkowitsch [1913], who found iodine values up to 145.

We examined the iodine value of cholesterol and of yeast sterol by the methods both of Hübl and of Wijs. With Wijs' solution the reaction is not confined to addition of halogen to the ethylene linkages and high values result. The acetates of the sterols behave similarly so that the alcohol group does not appear to be concerned in these changes. The presence of an appreciable quantity of sterols in any fat leads therefore to an abnormal value when determined by Wijs' reagent, and in such cases the iodine value cannot be taken as a measure of the number of ethylene linkages present in the molecule.

With Hübl's reagent the action with the sterol proceeds slowly and gradually, and falls off very much after 24 hours. Lewkowitsch found for the iodine value of cholesterol by Hübl's method the values 68.09 and 67.43. Theory for one unsaturated linkage in the molecule requires 62.8 corresponding to the formula  $C_{27}H_{46}O$ ,  $H_2O$ . We found values of from 65 to 75 which correspond approximately with this theoretical requirement. Werner found by Hübl's method for phytosterol values from 41 to 76, according to the time of the reaction; after half-an-hour by Wijs' method 135.

We then tested a sample of brassicasterol supplied to us by Professor Windaus and found for this with Hübl's reagent an iodine value of 118; theory for two double bonds demands 122. Windaus and Welsch [1909] have shown that brassicasteryl acetate adds on four atoms of bromine and contains

therefore two double bonds, a result with which the Hübl iodine value is in good agreement. Yeast sterol and its acetate under similar conditions give iodine values corresponding to the presence of three double bonds in the molecule. This result is in agreement with the formula ascribed to ergosterol by Tanret,  $C_{27}H_{42}O$ ,  $H_2O$ , a formula differing from that of cholesterol only in that it contains four hydrogen atoms less.

The evidence so far available (see Table III) therefore indicates that yeast sterol differs from the cholesterol and phytosterols yet described in that it contains three double bonds in the molecule.

Table III. Iodine Values of Sterols.

Substance and formula	I value (Wijs)	Time of reaction hrs.	I value (Hübl)	Time of reaction hrs.
<i>Cholesterol</i> $C_{27}H_{46}O$ , $H_2O$ [I value calc. if one double bond is present = 62.8]	141.6 152.7 165.1 165.8 153.2 158.4 165.4	6 18 22.5 22.5 24 25 48	58.65 70.21 64.88  73.2  71.8	6 18 22.5  24  48
<i>Cholesteryl acetate</i> [I value calc. if one double bond is present = 59.8]	120.2 127.8 131.1 144.1	6 18 24 48	27.94 48.26 49.8 52.1 62.6	6 18 20 24 48
<i>Brassicasterol</i> $C_{28}H_{46}O$ , $H_2O$ [I value calc. if two double bonds are present = 122.0]			118	22.5
<i>Sterol from yeast</i> , $C_{27}H_{42}O$ , $H_2O$ [I value calc. if three double bonds are present = 190.5]	337.8	23	177.1	21
<i>Steryl Acetate from yeast</i> [I value calc. if three double bonds are present = 179.1]	245.2	23	175.1	

*Probable identity of Mycosterol with Ergosterol.*

Recently Ikeguchi [1919] isolated a sterol from various fungi, *Armillaria edodes*, *Collybia shiitake*, *Hydnum asparatum* and *Lycoperdon gemmatum*, to which he gave the name of mycosterol. Its properties closely resemble those of ergosterol. It gives similar colour reactions when treated with chloroform and concentrated sulphuric acid, the red colour is in the acid layer, and its melting point, rotation and the melting point of its acetate are all in close agreement with those of ergosterol (see Table II). The difference in the analytical numbers may be due to the fact that Ikeguchi apparently analysed the crystals after recrystallising them from alcohol, when they contain one molecule of water of crystallisation, whereas other observers state that they dried to a constant weight over sulphuric acid.

Ikeguchi's numbers agree fairly with those required for the hydrated sterol. The percentage of carbon is low but this is possibly due to slight loss of water of crystallisation. Ikeguchi shows that only one acetyl group is introduced into the molecule on acetylation, and that a carbonyl group is

not present. He does not however consider the possibility of the second oxygen atom being present as water of crystallisation.

The only property of mycosterol which is not in agreement with our observations on yeast sterol is its behaviour on bromination. When bromine in acetic acid was added to an ether solution of mycosterol the crystals recovered agreed in melting point and in colour reactions with the original substance, and the conclusion is drawn that no double bond is present in the molecule. When a similar experiment was performed with yeast sterol, the bromine solution was rapidly decolorised and a black oily product was obtained on evaporating the solution, which solidified on standing, but we had not sufficient material to isolate a pure substance from this. In view however of the close agreement of mycosterol with ergosterol, further investigation of its behaviour on bromination seems desirable.

We are at present continuing our investigations on the constitution of yeast sterol.

#### SUMMARY AND CONCLUSIONS.

1. Palmitic, oleic and linoleic acids have now been identified with certainty in yeast fat.
2. The pentadecic acid previously described consists of a mixture of palmitic and lauric acids. Evidence for the presence of lauric acid has been obtained.
3. The presence of an acid melting at  $77^{\circ}$  has been confirmed, the melting point of which agrees with that of arachidic acid.
4. No confirmation of the presence of the dodecenic acid described by Hinsberg and Roos was obtained and the possibility of this being a mixture of lauric with oleic and linoleic acids has not yet been excluded.
5. A sterol is present partly in the free state and partly as fatty acid esters; this may constitute 20 % of the total yeast fat.
6. The sterol present in yeast appears to be identical with the ergosterol isolated from ergot by Tanret.
7. The variation in melting point described by different observers seems to be due to differences in the purity of the substance, but no conclusive evidence of the presence of a second sterol in yeast has yet been obtained.
8. It seems probable that the mycosterol isolated by Ikeguchi from certain fungi is also identical with ergosterol and that ergosterol is characteristic of the whole group of cryptogams just as cholesterol is of the animal and phytosterol is of the higher plant kingdom.
9. Yeast sterol is differentiated from the sterols of the higher plants and animals by the presence of three double bonds in its molecule.

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